I. Summary

Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by a deficiency in the receptor that clears low density lipoprotein (LDL) from the serum (reviewed in Ref. 1 and 2). Patients with one abnormal LDL receptor allele have moderate elevations in plasma LDL and suffer premature coronary heart disease. Approximately 5% of all patients under 45 who have had a myocardial infarction carry this trait. Patients with two abnormal LDL receptor genes (homozygous deficient patients) have severe hypercholesterolemia and life-threatening coronary artery disease in childhood.

Strategies for treating patients with FH are directed at lowering the plasma level of LDL. In heterozygotes, this is accomplished through the, administration of drugs that stimulate the expression of LDL receptor from the normal allele (2). This therapeutic approach is not effective in the treatment of homozygous deficient patients, especially those that retain <2% of residual LDL receptor activity. Partial amelioration of hyperlipidemia has been achieved in some homozygous deficient patients by diverting the portal circulation through a portacaval anastomosis (3) and by chronic plasmapheresis therapy (4). A more direct approach has been to correct the deficiency of hepatic LDL receptor by transplanting a liver that expresses normal levels of LDL receptor. Three patients that survived this procedure normalized their serum LDL-cholesterol (5-9).

We have used an authentic animal model for FH, the Watanabe Heritable Hyperlipidemic rabbit (WHHL), to develop gene therapies for the homozygous form of FH (10-13). The WHHL rabbit has a mutation in its LDL receptor gene which renders the receptor completely dysfunctional (12) leading to severe hypercholesterolemia, diffuse atherosclerosis, and premature death. The potential efficacy of gene therapy for FH is supported by a series of studies we have performed in the WHHL rabbit in which we have achieved metabolic improvement (14-l8). Liver tissue was removed from WHHL rabbits and used to isolate hepatocytes and establish primary cultures. A functional rabbit LDL receptor gene was transduced into a high proportion of hepatocytes using recombinant retroviruses, and the genetically corrected cells were transplanted into the animal from which they were derived. Transplantation of the genetically corrected, autologous hepatocytes was associated with a 30-40% decrease in serum cholesterol that persisted for the duration of the experiment (4 months, Ref. 18). Recombinant derived LDL receptor RNA was detected in liver for at least 6 months. There was no apparent immunological response to the recombinant derived LDL receptor (18).

Another study of relevance to the proposal involves the isolation and retrovirus mediated gene transfer of human hepatocytes (19). We have successfully isolated viable human hepatocytes from 4 donors and have achieved gene transfer in a large proportion of cells. Hepatocytes transduced with a gene encoding LDL receptor express levels of functional protein that exceed normal endogenous levels.

Based on our preclinical studies, we propose a protocol to treat homozygous FH patients by *ex vivo* gene therapy. The patient population we plan to treat are FH homozygotes with symptomatic CAD who have a relatively poor prognosis but can tolerate a noncardiac surgical procedure with acceptable risks. Both children and adults will be eligible for this therapy. Patients will be evaluated over a six week period to determine their eligibility in the study and to establish metabolic baselines. The proposed therapy will be an adjunct to the more traditional therapies such as plasma exchange and drugs which will be reinstituted 6 weeks after gene therapy. Eligible patients will be admitted to the hospital and subjected to a two step procedure in which a portion of liver is removed on day 0 and hepatocytes are isolated and plated in culture. Recombinant retroviruses will be used to transduce a normal LDL receptor gene into

the cultured hepatocytes which will be harvested on day 3 and infused into the portal circulation of the patient through an indwelling catheter.

The patient will be evaluated for engraftment of corrected hepatocytes through a series of metabolic studies. Three months after gene therapy, a small amount of liver tissue will be harvested by percutaneous biopsy and analyzed for the presence of recombinant derived RNA and DNA.